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### (54) Sugar super-tolerant yeast for confectionery and bakery

(57) The invention provides (1) yeasts with high leavening ability enough for bakery in an ultra-high sugar range and with high scar tolerance (osmotic pressure tolerance), and (2) yeasts with high leavening ability enough for bakery in an ultra-high sugar range and with high sugar tolerance (osmotic pressure tolerance) and

high freezing tolerance which characteristically exert sufficient leavening ability of dough blended with sugar above 40 parts by weight per 100 parts by weight of flour, to enable the production of high-quality bread items including white bread in a low sugar range to sweet roll items or fermented confectioneries in a high sugar range, irrespective of the bakery process.

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**Description**Field of the Invention

5 [0001] The present invention relates to novel yeasts and the use thereof.

Description of the Related Art

10 [0002] Bread is grouped in a wide variety of items, including French bread with no sugar added, white bread in a low sugar range, which is prepared by adding sugar at 5 to 6 % to flour, croissant and butter roll in a medium sugar range, which are prepared by adding sugar at 8 to 15 % to flour, Danish pastry and confectionery bread in a high sugar range, which are prepared by adding sugar at 20 to 30 % to flour, fermented confectioneries, such as bean-jam sweet, and coffee cake and brioche, which are prepared by adding sugar at 35 to 50 % to flour. Furthermore, bakery process is divided in scratch process (including straight bakery process and intermediate seed bakery process) and frozen dough process. Therefore, these processes and bread items are used in such a wide variety of combinations.

15 [0003] For producing the bread items in the low sugar range to the high sugar range, conventionally, various yeasts with various fermentative abilities have been used in such a complicated manner that yeast with high freezing tolerance should be used for the frozen dough process. However, no yeast with sugar tolerance enough for fermentation of dough items [for bean-jam sweet roll, fermented confectioneries, etc.] in a blend of sugar at a content as high as 35 % or more to flour has yet been present. Accordingly, many bakers have managed to treat such dough items by prolonging the duration of the fermentation more than necessary or raising the quantity of yeast, to prepare the products. Nevertheless, these products are currently of no bread quality primarily intended and have poor bulky appearance.

20 Problems that the Invention is to Solve

25 [0004] It is an object of the invention to provide yeasts with high sugar tolerance (or osmotic pressure tolerance) having high excellent fermentative abilities in a super-high sugar range, so as to solve the problems. In current circumstances where sweet bread and confectioneries with high sugar contents are desired in general, more specifically, the invention has been attained for the purpose of providing novel yeasts with super-high sugar tolerance, which conventionally have never existed and are hardly affected (of which the fermentative abilities are hardly reduced) by the osmotic pressure of high contents of fat or fatty oil or egg blended in dough at a super-high sugar content of 30 to 40 % or more. Conventional yeasts under the influence of osmotic pressure can never yield bread products of excellent quality from dough in a blend of fat or fatty oil or egg at a super-high sugar content of 30 to 40 % or more. Owing to the progress in bakery technology and the diversification of bread items in recent years, yeast should be selected and used, depending on the intended bread item. Thus, many types of yeast are essentially needed. In terms of laborious works in the use of a different yeast each time, which is not practical industrially, novel yeasts with excellent properties are provided, from the respect that one yeast concurrently having these properties can satisfy the whole aspect.

30 [0005] In other words, the invention provides excellent yeasts with great specificity from the respect of super-high sugar tolerance. Additionally, the invention can firstly provide new useful yeasts satisfying all the needs, which has never been obtained conventionally and which does not require the proper use of yeasts having different properties, depending on the intended products.

Means for Solving the Problems

45 [0006] The invention relates to yeasts with high sugar tolerance (or osmotic pressure tolerance). Specific characteristic properties of the inventive yeasts are as described below.

- 50 1. The yeasts have a leavening ability of dough blended with sugar at 5 to 25 % to flour for bread loaf and confectionery bread (in the low to high sugar range), at the same level as that of routine general types of yeast (for example, Regular Yeast manufactured by Oriental Yeast Co., Ltd.), at the straight dough method and the sponge dough method, to produce bread of large volume and stable quality.
2. The yeasts have sugar tolerance (osmotic pressure tolerance) capable of fermenting dough at a very high sugar concentration, such as dough containing sugar at 30 to 50 % to flour.
- 55 [0007] Of course, the presentive yeasts may be used to make variants breads and sweet rolls by using dough containing other sugar in place of sucrose; examples of said other sugar are glucose, fructose, liquid inverted sugar, etc.
- [0008] Various methods can be adopted so as to obtain the inventive yeasts. By the mating method, for example, the intended strain can be obtained highly efficiently. Firstly, therefore, strains with high fermentative abilities and with

a character raising sugar tolerance are selected among bakers' yeast strains of the individual yeast strains are inoculated on a spore forming culture medium in a conventional manner to form the spores; after the examination of the properties of the resulting spores, then, strains with the same bakery performance as general practical yeasts for bread making and also with a high leavening ability of 40 %- sugar blended dough is satisfactorily screened among strains grown by the traditional mating method. It is needless to say than the intended strains may be created by a mutation process. As the mutation process, routine methods can be adopted widely, including physical processes with  $\gamma$  ray, ultraviolet ray and temperature difference, etc. and processes with mutagens such as eihidium bromide, nitrogen mustard, diepoxybutane, colochicine, peroxide, and purine derivatives.

[0009] One of the new yeast strains thus obtained was designated *Saccharomyces cerevisiae* P-712 and deposited under FERM BP-7034 at National Institute of Bioscience and Human-Technology Agency of Industrial Science and Technology. The bacteriological properties are described below.

#### Bacteriological properties of the strain P-712

15 1. Growth state

[0010] Good growth in YM liquid culture medium.

[0011] Cell morphology: spherical to egg shape; 3 to 7  $\times$  4 to 8  $\mu$ . MM agar culture medium: good growth; colony (white, glossy, and smooth).

20 2. Ascospore

[0012] Formed in potassium acetate culture medium; ascospore of spherical shape.

25 3. Individual physiological properties

[0013]

- 30 3-1. Optimum growth conditions: temperature of 28 to 32 °C; pH 4.5 to 6.5.
- 3-2. Growth range: temperature of 5 to 40 °C; pH 2.5 to 8.0.
- 3-3. Assimilation of nitrate salt: none.
- 3-4. Fat decomposition: none.
- 3-5. Carotenoid generation: none.
- 3-6. Prominent organic acid generation: none.
- 35 3-7. Vitamin requirement: biotin and pantothenic acid.
- 3-8. Sugar tolerance: sufficient leavening ability can be exerted in dough blended with sucrose above 30 to 40 parts by weight or more to 100 parts by weight of flour.

40 4. Fermentability and assimilability of carbon source

[0014]

	Fermentability	Assimilability
D-Glucose	+	+
D-Galactose	+	+
Maltose	+	+
Sucrose	+	+

50 [0015] In accordance with the invention, furthermore, a new yeast strains are provided, having a high leavening ability in a super-high sugar range as well as freezing tolerance.

[0016] Various methods can be adopted so as to obtain the inventive yeasts. By the mating method, for example, the intended strain can be obtained highly efficiently. Firstly, therefore, strains with high fermentative abilities and with a character raising both freezing tolerance and sugar tolerance are selected among bakers' yeast strains of the individual yeast strains are inoculated on a spore forming culture medium in a conventional manner to form the spores; after the examination of the properties of the resulting spores, then, strains with the same bakery performance as general practical yeasts for bread making and also with a high leavening ability of 40 %- sugar blended dough and

freezing tolerance is satisfactorily screened among strains grown by the traditional mating method. It is needless to say than the intended strains may be created by a mutation process. As the mutation process, routine methods can be adopted widely, including physical processes with  $\gamma$  ray, ultraviolet ray and temperature difference, etc. and processes with mutagens such as ethidium bromide, nitrogen mustard, diepoxybutane, colchicine, peroxide, and purine derivatives.

5 [0017] One of the new yeast strains was designated *Saccharomyces cerevisiae* P-731 and deposited under FERM BP-7035 at National Institute of Bioscience and Human-Technology Agency of Industrial Science and Technology. The bacteriological properties are described below.

10 Bacteriological properties of the strain P-731

1. Growth state

15 [0018] Good growth in YM liquid culture medium.  
 [0019] Cell morphology: spherical to egg shape; 3 to 7  $\times$  4 to 8  $\mu$ . MM agar culture medium: good growth; colony (white, glossy, and smooth).

2. Ascospore

20 [0020] Formed in potassium acetate culture medium; ascospore of spherical shape.

3. Individual physiological properties

[0021]

25 3-1. Optimum growth conditions: temperature of 28 to 32 °C; pH 4.5 to 6.5.  
 3-2. Growth range: temperature of 5 to 40 °C; pH 2.5 to 8.0.  
 3-3. Assimilation of nitrate salt: none.  
 3-4. Fat decomposition: none.  
 30 3-5. Carotenoid generation: none.  
 3-6. Prominent organic acid generation: none.  
 3-7. Vitamin requirement: biotin and pantothenic acid.  
 3-8. Freezing tolerance/sugar tolerance: after thawing of the frozen dough containing 40 parts by weight of sucrose per 100 parts by weight of flour (under 4-week storage), the strain generates CO<sub>2</sub> 100 ml or more per 40 g of the  
 35 dough containing 0.4 g yeast on a dry weight basis at 30 °C for 2 hours.

4. Fermentability and assimilability of carbon source

[0022]

40

	Fermentability	Assimilability
D-Glucose	+	+
D-Galactose	+	+
Maltose	+	+
Sucrose	+	+

45

[0023] The above-mentioned two yeast strains deposited are considered to belong to *Saccharomyces cerevisiae*; the strains have the bacteriological properties described above, but the strains have high fermentation potencies in the super-high sugar range and also in the low to medium sugar range. Such strains have never been found among conventionally known yeast strains. Hence, the two yeast strains are designated new strains. Furthermore, not only the above two new strains are encompassed within the scope of the invention; but also all yeast strains having the properties described above, including artificially created strains and strains from the natural origin, are encompassed within the scope of the invention.

55

(1) The inventive yeast strains with high leavening abilities in the super-high sugar range may be selected and grown in the manner described below. They with the sugar tolerance can be obtained, by separating each yeast strain

generating CO<sub>2</sub> 110 ml or more at 30 °C for 2 hours, per 40 g of dough containing 0.4 g of said each yeast strain on a dry weight basis containing sucrose of 40 parts by weight per 100 parts by weight of flour. The inventive strains are sometimes referred to as "US yeast".

5 (2) The inventive yeast strains with a high leavening ability in the super-high sugar range and freezing tolerance may be selected and grown in the manner described below. After the thawing of frozen dough containing 40 parts by weight of sucrose to 100 parts by weight of flour, each yeast strain generating CO<sub>2</sub> 100 ml or more at 30 °C for 2 hours per 40 g of the frozen dough containing 0.4 g of said each yeast strain on a dry weight basis is satisfactorily separated. The inventive strains are hereinafter sometimes referred to as "FD-2 yeast".

10 [0024] In accordance with the invention, occasionally, the aforementioned two yeast strains (1) and (2) are collectively called "sugar super-tolerant yeast strain".

[0025] Owing to the excellent sugar tolerance capable of overthrowing the state of art, the sugar super-tolerant yeast can be used widely for the production of bread items with no sugar to bread items in a low sugar range, like French bread and white of bread, as well as confectionery bread to fermented confectioneries prepared from dough at super-high sugar contents, and also for the production of various bread items using fat or fatty oil or egg or the like. The sugar super-tolerant yeast can be used, for example, for producing coffee cake (bread suitable with coffee, which is preferred by many Americans), bean-jam sweet roll, fruit bread, pastry, butter roll and croissant.

15 [0026] The process of producing bread using the yeast of the present invention is satisfactorily any scratch process (straight dough method and sponge dough method) in a conventional manner and is applicable to frozen bread dough in addition to general bread dough. Examples of the invention are described below.

#### Example 1

20 [0027] The inventive sugar super-tolerant yeast strain (FERM BP-7034) was cultured under the following conditions, using a 30-liter jar fermenter, to produce much thereof.

Seed culture	
Sucrose content (on a glucose basis)	1035 g
Urea	103 g
Monosodium phosphate · dihydrate	20.7 g
Quantity of seed yeast (wet basis)	20g *1
30-liter jar culture	
Sucrose content (on a glucose basis)	1400 g
Urea	140 g
Monosodium phosphate · dihydrate	28 g
Quantity of seed yeast (wet basis)	420g *2

40 Maker: Oriental Bio-Service Co., Ltd.  
Name: Fermenter CONTROL SYSTEM MC-10

Volume: 30 liters

Agitation: 600 rpm

Aeration volume: 16 liters/min

\*1 One platinum loop of the seed was inoculated in a 250-ml YPD culture medium/1-liter Sakaguchi's flask for 2-day culture at 30 °C; the total volume in the 4 flasks of themselves was used as seed yeast.

45 \*2 The cell obtained by the seed culture were separated by centrifugation and rinsed in deionized water, and a part thereof was used.

#### Example 2

50 [0028] According to the assay method of the gas generated by yeast, as defined by Japan Yeast Industry Association, the sugar tolerance of each yeast was assayed. By using the inventive yeast (sugar super-tolerant yeast) FERM BP-7034 and a commercially available regular yeast (product manufactured by Oriental Yeast Co., Ltd.), more specifically, the gas generated from the following blends at 30 °C for 120 minutes was assayed under the following conditions by Fermograph. The results are shown in Table 2.

Table 1

Formula	0	5	10	15	20
Flour	100	100	100	100	100
Sucrose	0	5	10	15	20
Sodium chloride	0.5	0.5	0.5	0.5	0.5
Yeast	6	6	6	6	6
Water	68	65	62	60	58

Formula	25	30	35	40	45
Flour	100	100	100	100	100
Sucrose	25	30	35	40	45
Sodium chloride	0.5	0.5	0.5	0.5	0.5
Yeast	6	6	6	6	6
Water	56	54	52	50	48

Formula	50
Flour	100
Sucrose	50
Sodium chloride	0.5
Yeast	6
Water	46

Process conditions

## [0029]

Mixer: Hobart mixer

Mixing: low speed for 3 minutes

Kneading temperature: 30 °C

Fermentation temperature: 30 °C

Assay: total gas volume from dough weight of 40 g at 30 °C for 2 hours assayed by Fermograph (manufactured by Atto Co., Ltd.).

Table 2

Comparison of leavening abilities		
(unit: ml/40g · dough/2 hr)		
Sucrose content	Inventive US yeast	Regular yeast
0	219	220
5	301	303
10	330	328
15	325	325
20	301	295
25	275	235
30	235	171
35	175	114

Table 2 (continued)

Comparison of leavening abilities		
(unit: ml/40g · dough/2 hr)		
Sucrose content	Inventive US yeast	Regular yeast
40	134	75
45	80	38
50	45	18

The quantity of yeast contained in 40 g of the dough corresponds to 0.40 g on a dry weight basis.

[0030] As apparently shown in the results of Table 2, it was confirmed that the sugar super-tolerant yeast of the invention still retained sugar tolerance (osmotic pressure tolerance) capable of sufficiently fermenting dough at a very high sugar concentration, such as dough containing sucrose at 30 to 50 % to flour.

### Example 3

[0031] Using the inventive US yeast (FERM BP-7034), mini-type bean-jam sweet roll was prepared from the following blend, by the following process; Table 3 and 4.

Table 3

Formula	(Formula ratio per flour part by weight)	
	Sponge	Dough mixing
Flour	70.0	30.0
"US yeast"	4.0	1.0
"Saka-rich"	15.0	
Dough improve	0.5	0.2
Sugar	(Glucose) 5.0	(Sucrose) 5.0
Shortening		10.0
Skim milk powder		3.0
Sucrose		40.0
Sodium chloride		0.35
Whole egg	10.0	
Water	19.0	

Table 4

Process conditions		
	Sponge	Dough mixing
Mixing time (minutes)	L2M2	L2M3↓M3H1
Dough temperature	26 °C	28 °C
Fermentation (floor)	2 hours	90 minutes
Fermentation Conditions	28 °C	28 °C
Dividing weight		15 g
Bench time		15 minutes

Table 4 (continued)

Process conditions		
	Sponge	Dough mixing
5 Bean-jam weight (per dough)		10 g
Final proof condition		35 °C · 85 % RH about 70 minutes
Baking conditions		200 °C for 8 minutes

10 Example 4

[0032] Using the inventive US yeast, roll with sucrose was prepared from the following blend at the following conditions; Table 5 and 6. At a sucrose content of 15 to 25 parts by weight, the US yeast was effective, advantageously.

15 Table 5

(Formula ratio per flour part by weight)		
Formula		
	Sponge	Dough mixing
Flour	70.0	30.0
"US yeast"	3.0	
Dough improve	0.1	
Sugar (Glucose)	3.0	(Sucrose) 20.0
Shortening		10.0
Skim milk powder		3.0
Sodium chloride		1.0
Whole egg	10.0	
Water	34.0	20.0

35

Table 6

Process conditions		
	Sponge	Dough mixing
40 Mixing time (minutes)	L2M2	L2M3↓M3H1
Dough temperature	26 °C	28 °C
Fermentation (floor)	2 hours	50 minutes
45 Fermentation Conditions	28 °C	28 °C
Dividing weight		60 g
Bench time		15 minutes
Final proof condition		35 °C · 85 % RH about 60 minutes
50 Baking conditions		200 °C · 9 minutes

Example 5

[0033] Using the inventive US yeast, fruit bread was prepared from the following blend at the following conditions; Table 7 and 8. The resulting product was a confectionery-like bread item at a high content of fruit used therein and with fluffy taste; owing to the combined use of the fermentation flavor liquid "Budou Dane #2" (product of Oriental Yeast Co., Ltd.), the resulting bread was very flavorful and delicious.

Table 7

(Formula ratio per flour part by weight)		
Formula	Sponge	Dough mixing
Flour	70.0	30.0
"US yeast"	3.0	
"Budou Dane #2"	5.0	
Dough improve	0.1	
Sugar	(Glucose) 3.0	(Sucrose) 20.0
Fats and fatty oils (shortening)		10.0
Sodium chloride		1.5
Whole egg	10.0	
Skim milk powder		3.0
Raisin		70.0
Water	30.0	14.0

Table 8

Process conditions		
	Sponge	Dough mixing
Mixing time (minutes)	L2M2	L2M3↓M3H1↓L3
Dough temperature	26 °C	28 °C
Fermentation (floor)	2 hours	60 minutes
Fermentation Conditions	28 °C	28 °C
Dividing weight		60 g
Bench time		15 minutes
Molding		
Final proof condition		35 °C · 85 % RH about 60 minutes
Baking conditions		200 °C · 10 minutes

## Example 6

- 45 [0034] Using the inventive US yeast, a fermented confectionery (cookie) was prepared from the following blend at the following conditions; Table 9. The resulting product was very sweet with fluffy touch on teeth.

Table 9

(Formula ratio per flour part by weight)	
Formula	
Flour	100.0
"US yeast"	5.0
Sucrose	40.0
Margarine	40.0

Table 9 (continued)

(Formula ratio per flour part by weight)	
Formula	
Whole egg	10.0
Condensed milk	0.5
Vanilla oil	0.3
Water	10.0
Sodium chloride	0.5

Process conditions

## [0035]

1. Mixed sucrose, margarine, condensed milk and sodium chloride with a beater.
2. Add egg in a two-divided manner and emulsify the egg.
3. Add sifted flour, US yeast, and water and mix the resulting mixture together.
4. Gently mixed the mixture with hands.
5. Place the dough in a polyethylene bag and elongate flat wise the dough with hands; cool the dough in a refrigerator to a final stiffness easily moldable enough for molding and punch it.
6. Ferment the dough at room temperature for 20 to 30 minutes and bake the dough at 220°C for about 13 minutes.

## Example 7

[0036] Using the inventive US yeast and a commercially available regular yeast (manufactured by Oriental Yeast Co., Ltd.), a white bread and a sweet roll item were prepared from the following formulas at the following conditions; Table 10 and 11, according to the scratch bakery process. The results are shown in Table 12.

Table 10

(Formula ratio per flour part by weight)		
Formula		
	Low sugar content (white bread) dough blend	High sugar content (sweet roll) dough blend
Flour	100.0	100.0
Sucrose	5.0	25.0
Sodium chloride	2.0	0.5
Yeast	2.0	3.0
Shortening	5.0	6.0
Skim milk powder	0.0	2.0
Water	65.0	52.0

Table 11

Process conditions		
	Low sugar content (white bread) dough blend	High sugar content (sweet roll) dough blend
Mixing time (minutes)	L2M2↓L2M2H2	L2M4↓L1M4H
Dough temperature	28 °C	28 °C
Dividing weight	450 g	60 g

Table 11 (continued)

Process conditions			
	Low sugar content (white bread) dough blend	High sugar content (sweet roll) dough blend	
5	Bench conditions	30 °C for 15 minutes	30 °C for 15 minutes
10	Molding	one loaf	molded into sweet roll
	Baking conditions	200 °C for 25 minutes	200 °C for 10 minutes

Table 12

Comparison by the scratch process		
Straight process	Inventive US yeast	Regular yeast
Whit bread		
First fermentation conditions	30 °C for 73 minutes	30 °C for 75 minutes
Final proof conditions	38 °C for 56 minutes	38 °C for 57 minutes
Specific bread volume	4.38	4.32
Bread quality (softness)	soft	ordinary
Bread quality (aging)	slow	ordinary
Bread quality (texture)	uniform	ordinary
Bread flavor	good	good
Texture	soft	ordinary
Sweet roll		
First fermentation conditions	76 minutes	81 minutes
Final proof conditions	38 °C for 55 minutes	38 °C for 56 minutes
Specific bread volume	5.21	5.09
Bread quality (softness)	soft	ordinary
Bread quality (aging)	slow	ordinary
Bread quality (texture)	uniform	ordinary
Bread flavor	good	good
Texture	soft	ordinary

[0037] In the same manner, subsequently, a white bread was prepared according to the scratch process (sponge dough method) from the following formula and under the following conditions. The results are shown below in Table 13.

Table 13

(Formula ratio per flour part by weight)			
	70 % intermediate seed bakery process (bread loaf)		
50	Formula	Sponge	Dough mixing
	Flour	70.0	30.0
55	Sucrose		5.0
	Sodium chloride		2.0
	Yeast	2.0	

Table 13 (continued)

(Formula ratio per flour part by weight)		
	70 % intermediate seed bakery process (bread loaf)	
Formula	Sponge	Dough mixing
Dough improver (Ammonium chloride, calcium carbonate)	0.1	
Water	38.5	25.0

Table 14

Process conditions		
	Sponge	Dough mixing
Mixing time (minutes)	L1 M2	L1M6H3
Dough temperature	24 °C	28 °C
Fermentation conditions	30 °C 28 °C for 4 hours	30 °C for 15 minutes
Dividing weight		450 g
Fermentation (floor)		15 minutes or 30 minutes
Bench time		15 minutes
Final proof		35 °C · 85 % RH
Baking conditions		200 °C for 20 minutes

Table 15

Comparison by the scratch process		
Sponge dough method	Inventive US yeast	Regular yeast
Sponge leavening volume for 1 hr	145 ml	120 ml
Dough leavening volume for 1 hr	315 ml	300 ml
Final proof	35 °C for 52 minutes	35 °C for 55 minutes
Specific bread volume	6.0	5.8
Bread quality (softness)	soft	ordinary
Bread quality (aging)	slow	ordinary
Bread quality (texture)	uniform	ordinary
Bread flavor	good	good
Texture	soft	ordinary

[0038] As apparently shown above in the results of Table 15, it was confirmed that the inventive US yeast had a leavening ability at the same level as that of regular yeast (for example, Regular Yeast manufactured by Oriental Yeast Co., Ltd.) and could produce excellent bread of stable quality and softness and with large volume, from dough blended with sugar at 5 to 25 % to flour (in the low to high sucrose range) for white bread and sweet roll, according to any of the straight bakery method and the sponge dough method.

## Example 8

[0039] The inventive FD-2 yeast (FERM BP-7035) was cultured and prepared on a large scale, using a 30-liter jar fermenter under the following conditions.

Seed culture		
5	Sugar content (on a glucose basis)	1035 g
	Urea	103 g
	Monosodium phosphate · dihydrate	20.7 g
	Quantity of seed yeast (wet basis)	20g *1
10	30-liter jar culture	
	Sugar content (on a glucose basis)	1400 g
	Urea	140 g
	Monosodium phosphate · dihydrate	28 g
	Quantity of seed yeast (wet basis)	420g *2

15 Maker: Oriental Bio-Service Co., Ltd.  
 Name: Fermenter CONTROL SYSTEM MC-10  
 Volume: 30 liters  
 Agitation: 600 rpm  
 Aeration volume: 16 liters/min  
 \*1 One platinum loop of the seed was inoculated in a 250-ml YPD culture medium/1-liter Sakaguchi's flask for 2-day culture at 30°C; the total volume in the 4 flasks of themselves was used as seed yeast.

20 \*2 The cells obtained from the seed culture were separated by centrifugation and rinsed in deionized water; and a part thereof was used.

#### Example 9

25 [0040] The freezing tolerance was compared between the inventive yeast (P-731 FERM BP-7035: FD-2 yeast) and a commercially available freezing tolerant yeast (without sugar tolerance) (FD-1 yeast: product of Oriental Yeast Industry, Co., Ltd.) in various blends with sugar.

30 [0041] More specifically, the inventive yeast prepared as described above was added to and mixed and kneaded with bread dough at the following Table 16. After mixing and kneading, the resulting dough was divided into 40-g portions, which were then fermented at 30°C and molded thereafter; then, the molded portions were frozen and stored for given periods of time at -20°C. As a control, the commercially available freezing tolerant yeast was used, to prepare such frozen dough in the same manner, for storage.

Table 16

	(Formula ratio per flour part by weight)				
	0	5	10	15	20
35	Flour	100	100	100	100
	Sucrose	0	5	10	15
	Sodium chloride	0.5	0.5	0.5	0.5
	Yeast	6	6	6	6
	Water	68	65	62	60

	(Formula ratio per flour part by weight)			
	25	30	40	50
45	Flour	100	100	100
	Sucrose	25	30	40
	Sodium chloride	0.5	0.5	0.5
	Yeast	6	6	6
	Water	56	54	50

#### Process conditions

55 [0042]

Mixer: Hobart mixer  
 Mixing: low speed for 3 minutes

Dough temperature: 24 °C  
 Quantity of divided dough portion: 40 g  
 Fermentation prior to dough freezing (floor time): 15 minutes (28 °C)  
 Dough freezing: -40 °C for 30 minutes  
 Storage of frozen dough: -20 °C  
 Thawing: 20 °C for 60 minutes  
 Assay: total gas volume from dough weight of 40 g at 30 °C for 2 hours was assayed by Fermograph (manufactured by Atto Co., Ltd.).

10

Table 17

Comparison of fermentability							
(mg/40g · dough/2 hr)							
		Inventive FD-2 yeast			Commercially available freezing tolerant yeast		
Period of under freezing	dough storage	1 day	4 weeks	8 weeks	1 day	4 weeks	8 weeks
sucrose content	0%	205	185	155	210	192	156
	5%	262	248	224	265	252	221
	10 %	292	272	251	288	269	249
	15%	280	260	243	279	255	241
	20%	258	243	230	256	239	228
	25%	237	225	211	231	218	205
	30%	204	192	180	195	182	169
	40%	118	107	103	85	66	45
	50%	47	40	35	28	19	12

[0043] As apparently shown Table 17 in the results, it was confirmed that the inventive freezing tolerant yeast (FD-2 yeast) had freezing tolerant at the same level as that of the commercially available freezing tolerance yeast and still had sugar super-tolerance capable of sufficiently fermenting dough at a high sugar concentration (even dough at a very high sugar concentration, such as dough containing sucrose at 30 to 50 % to flour).

## Example 10

[0044] Using the inventive FD-2 yeast (FERM BP-7035), coffee cake was prepared from the following blend at the following steps of the following bakery process (general bakery process: scratch process, frozen dough method).

Formula per flour part by weight		
Flour		100
Yeast (FD-2 yeast)		8
Dough improver		2
(*010* for frozen dough: product manufactured by Oriental Co., Ltd.)		
Sucrose		40
Margarine		25
Sodium chloride		0.8
Skim milk powder		6
Whole egg		25
Water		20
Flour paste (per dough)		60
(*Tokachi milk*: product manufactured by Oriental Co., Ltd)		

Process condition	
5	Mixing time (minutes) $L_2M_4H_6 \downarrow M_3H_{10}$
	Dough temperature 25 °C
	Floor time 40 minutes
	Retard temperature 5 °C
	Retard time 15 to 20 minutes
10	Roll-in being folded in four: once
	Dividing weigh 70 g
	Dough freezing - 40 °C for 30 minutes
	Dough storage under freezing - 20 °C for 6 weeks
	Thawing 20 °C for 60 minutes
15	Final proof 70 minutes
	Final proof conditions 35 °C · 85 % RH
	Baking conditions 200 °C for about 12 minutes

[0045] Because of the high content of sugar, coffee cake with a new taste, such as sweet taste and soft touch, like sponge cake was obtained by baking the dough after thawing, which was a brand-new bread product having new flavor and giving new texture.

[0046] Furthermore, blend doughs with addition of sucrose at 10 to 25 % to flour in the medium to high sugar range were individually prepared (100 g of flour, 10 to 25 g of sucrose, 0.5 g of sodium chloride, 6 g of yeast and 52 ml of water); the doughs were kneaded and divided into 30-g portions on a flour basis, which were preliminarily fermented at 30 °C for 30 minutes; each portion was molded, frozen and stored for 1 to 3 weeks; after thawing, the gas generated at 30 °C for 120 minutes was assayed by Fermograph. Consequently, it was verified that the inventive freezing tolerant/sugar super-tolerant yeast (FD-2 yeast) had freezing tolerance at the same level as that of standard yeast for frozen dough (for example, FD-1 yeast: product of Oriental Yeast Co., Ltd.), to produce high-quality bread even after the yeast was stored in frozen bakery dough for a long term.

#### Example 11

[0047] Frozen dough of the following Table 18 prepared by using the inventive FD-2 yeast at the following Table 19 was thawed, to prepare mini-type bean-jam sweet roll. Herein, "Saka-rich" is a fermentation flavor solution like "Sakadane". These are products of Oriental Yeast Industry, Co., Ltd.

Table 18

Formula (Formula ratio per flour part by weight)	
Formula	
40	Flour 100.0
	"FD-2 yeast" 5.0
	" Saka-rich" 15.0
45	Dough improve 1.0
	Sucrose 10.0
	Shortening 10.0
50	Skim milk powder 3.0
	Liquid inverted sugar 40.0

Table 18 (continued)

Formula (Formula ratio per flour part by weight)	
Formula	
Sodium chloride	0.35
Whole egg	10.0
Water	19.0

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Table 19

Process conditions	
Mixing time (minutes)	$L_2M_4H_6 \downarrow M_3H_{10}$
Dough temperature	25 °C
Fermentation (floor)	40 minutes
Fermentation temperature	28 °C
Dividing weight	15 g
Bench time	15 minutes
Bean-jam weight (per dough)	10 g
Dough freezing	- 40 °C for 30 minutes
Dough storage under freezing	- 20 °C for 4 weeks
Thawing:	20 °C for 60 minutes
Final proof condition	35 °C · 85 % RH about 70 minutes
Baking conditions	200 °C for about 8 minutes

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## Example 12

35 [0048] Sweet roll using sucrose was prepared, by using the inventive FD-2 yeast from the following Table 20 at the following Table 21. Even at a sucrose content of 15 to 25 parts by weight, the FD-2 yeast was effective.

Table 20

(Formula ratio per flour part by weight)	
Formula	
Flour	100.0
FD-2 yeast	3.0
Dough improve	1.0
Sucrose	23.0
Fats and fatty oils (shortening)	10.0
Skim milk powder	3.0
Sodium chloride	1.0
Whole egg	10.0
Water	54.0

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Table 21

Process conditions	
5	Mixing time (minutes) $L_2M_4H_6 \downarrow M_3H_{10}$
10	Dough temperature 25 °C
15	Fermentation (floor) 30 minutes
20	Fermentation temperature 28 °C
	Dividing weight 60 g
	Bench time 15 minutes
	Dough freezing - 40 °C for 30 minutes
	Dough storage under freezing - 20 °C for 5 weeks
	Thawing: 20 °C for 60 minutes
	Final proof condition 35 °C · 85 % RH about 60 minutes
	Baking conditions 200 °C for about 9 minutes

## Example 13

[0049] Fruit bread was prepared by using the inventive FD-2 yeast, after thawing the frozen dough of the following Table 22 as prepared at the following Table 23. The resulting product was a confectionery-like bread item at a high content of fruit used therein and with fluffy taste; owing to the combined use of the fermentation flavor liquid "Budou Dane #2" (product of Oriental Yeast Co., Ltd.) the resulting bread was very flavorful and delicious.

Table 22

Formula	
30	Flour 100.0
35	FD-2 yeast 3.0
40	"Budou Dane #2" 5.0
45	Dough improver 1.0
	Sucrose 25.0
	Shortening 10.0
	Sodium chloride 1.5
	Whole egg 10.0
	Skim milk powder 3.0
	Raisin 70.0
	Water 44.0

Table 23

Process conditions	
50	Mixing time (minutes) $L_2M_8 \downarrow M_5H_6 \downarrow L_8$
55	Dough temperature 26 °C
	Fermentation (floor) 60 minutes
	Fermentation temperature 28 °C
	Dividing weight 60 g

Table 23 (continued)

Process conditions	
Bench time	15 minutes
Molding	
Dough freezing	- 40 °C for 30 minutes
Dough storage under freezing	- 20 °C for 6 weeks
Thawing:	20 °C for 60 minutes
Final proof condition	35 °C · 85 % RH about 60 minutes
Baking conditions	200 °C for about 10 minutes

### 15 Example 14

**[0050]** Using the inventive FD-2 yeast, a fermented confectionery (cookie) was prepared from the frozen dough of the following Table 24 as prepared at the following process. The resulting product was a fermented confectionery, very sweet with fluffy touch on teeth.

Table 24

(Formula ratio per flour part by weight)	
Flour	100.0
FD-2 yeast	5.0
Sucrose	40.0
Margarine	40.0
Whole egg	10.0
Condensed milk	0.5
Vanilla oil	0.3
Water	10.0
Sodium chloride	0.5

### Process condition

[0051]

1. Mixed refined sucrose, margarine, condensed milk and sodium chloride with a beater.
  2. Add egg in a two-divided manner and emulsify the egg.
  3. Add sifted flour, FD-2 yeast and water and mix the resulting mixture together.
  4. Gently knead the mixture with hands.
  5. Place the dough in a polyethylene bag and flat wise and the dough with hands; cool the dough in a refrigerator to a final stiffness easily moldable enough for molding and punch it.
  6. Ferment the dough at room temperature for 20 to 30 minutes.
  7. Freeze the molded dough at - 40 °C for 30 minutes.
  8. Store the dough at -20 °C for 6 weeks.
  9. Thaw the dough at 20 °C for 60 minutes.
  10. Bake the dough at 220 °C for about 13 minutes.

### Example 15

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**[0052]** Using the inventive FD-2 yeast and a commercially available regular yeast (manufactured by Oriental Yeast Industry, Co., Ltd.), a white bread and a sweet roll item were prepared from the following Table 25 under the following conditions, according to the scratch bakery process (straight bakery process). The results are shown in Table 27.

Table 25

(Formula ratio per flour part by weight)		
	Low sugar content (white bread)	High sugar content (sweet roll)
5	Flour	100
10	Sucrose	5
15	Sodium chloride	2
20	Yeast	2
25	Shortening	5
30	Skim milk powder	0
35	Water	65
40		52

Table 26

Process conditions		
20	Mixing time (minutes)	L2M2↓L2M2H2
25	Dough temperature	28 °C
30	Dividing weight	450 g
35	Bench time	30 °C for 15 minutes
40	Molding	one loaf
45	Baking conditions	molded into sweet roll
50		200 °C for 25 minutes
55		200 °C for 10 minutes

Table 27

Comparison by the scratch bakery process		
35	Straight process	Inventive FD-2 yeast
40	White bread loaf: first fermentation	30 °C for 70 minutes
45	Final proof	30 °C for 75 minutes
50	Specific bread volume	38 °C for 58 minutes
55	Bread quality (softness)	38 °C for 57 minutes
35	Bread quality (aging)	4.42
40	Bread quality (texture)	4.32
45	Bread flavor	soft
50	Bread texture	ordinary
55	Sweet roll: first fermentation	slow
35	Final proof	ordinary
40	Specific bread volume	75 minutes
45	Bread quality (softness)	81 minutes
50	Bread quality (aging)	38 °C for 56 minutes
55	Bread quality (texture)	38 °C for 56 minutes
35	Bread flavor	5.30
40	Bread texture	good
45		good
50		ordinary
55		ordinary
35		uniform
40		ordinary
45		soft
50		slow
55		ordinary
35		good
40		good
45		soft
50		ordinary
55		ordinary

[0053] Additionally, it is verified from the aforementioned examples that the freezing tolerant, sugar super-tolerant yeast of the invention has an excellent leavening ability of non-frozen dough, to produce various bakery products. The inventive yeast exerted sufficient freezing tolerance and sugar super-tolerance in frozen dough, to prepare excellent bakery products from the frozen dough, even by the frozen dough process.

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## Example 16

[0054] Using the inventive P-731 strain (FERM BP-7035), Danish pastry was prepared from the following Table 28 under the following Table 29 according to the process of refrigerated dough in large portions. The resulting Danish pastry was highly suppressed of acid odor and fermentation smell and was greatly flavorful with butter and tasted very delicious. As described above, it was confirmed that the inventive yeast could be used for the refrigerated dough process.

Table 28

Pastry process conditions (Formula ratio per flour part by weight)	
Flour	100.0
Sucrose	20.0
Sodium chloride	1.0
Skim milk powder	3.0
Margarine	20.0
Whole egg	20.0
Dough improver	0.5
Yeast	6.0
Water	40.0
Roll-in fats and fatty oils	50.0

Table 29

Process conditions	
Mixing time (minutes)	$L_2M_3 \downarrow L_1M_3H_1$
Dough temperature	25 °C
Fermentation (floor)	60 minutes
Retard	
Dough weight in large portions	2 kg
Storage temperature (°C)	5 °C for 12 hours
Roll-in	Being folded in tree : thrice
Molding	square type
Final proof condition	35 °C -75 % RH 75 minutes
Baking conditions	210 °C for about 12 minutes

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Advantages of the Invention

[0055] In accordance with the invention, yeasts have been developed, having leavening abilities and high sugar tolerance in the ultra-high sugar range, and yeasts with a high leavening ability in the super-high sugar range and freezing tolerance in the ultra-high sugar range.

[0056] The inventive sugar super-tolerant yeasts are applicable widely to the production of from confectionery bread to fermented confectionery from dough formula of ultra-high sugar content, in addition to bread in the low sugar range,

and are additionally applicable to various bread items using fat or fatty oil or eggs, and are widely applicable to frozen dough process as well general bakery process. For example, the inventive yeasts can be used for producing coffee cake (bread suitable for coffee, which is popular among many Americans), sweet roll, fruit bread, pastry, butter roll, croissant and the like.

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**Claims**

1. Yeast with sugar tolerance generating CO<sub>2</sub> 110 ml or more per 40 g of dough containing 0.4 g the yeast on a dry weight basis, the dough being confectionery or bread dough containing 40 parts by weight of sucrose per 100 parts by weight of flour.
2. A biologically pure culture of Saccharomyces cerevisiae P-712, FERM BP-7034 with the sugar tolerance according to claim 1.
3. Yeast with character of claim 1 and freezing tolerance after thawing of the frozen dough (under 4-week storage) generating CO<sub>2</sub> 100 ml or more per 40g of dough containing 0.4 g parts yeast on a dry weight basis, the dough being confectionery or bread dough containing 40 parts by weight of sucrose per 100 parts by weight of flour at 30 °C for 2 hours.
4. A biologically pure culture of Saccharomyces cerevisiae P-731 ,FERM BP-7035 with the character of claim 1 and freezing tolerance according to claim 3.
5. Confectionery or bread dough containing the yeast according to claim 1.
6. Confectionery or bread dough containing the yeast according to claim 3.
7. A confectionery or a bread item prepared by fermenting the confectionery or bread dough according to claim 5 and subsequently baking the dough.
8. A confectionery or a bread item prepared by fermenting the confectionery or bread dough according to claim 6 and subsequently baking the dough.

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### PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention EP 00 40 0649  
shall be considered, for the purposes of subsequent  
proceedings, as the European search report

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	US 5 801 049 A (ENDO HISANORI) 1 September 1998 (1998-09-01) * tables 2,3 *	1-8	C12N1/18 A21D10/00 A21D13/00
A	TANAKA T. ET AL.: "Screening of Sugar-Tolerant Baker's Yeast for Sweet Buns" JOURNAL OF JAPANESE SOCIETY OF NUTRITION AND FOOD SCIENCE, vol. 31, no. 10, 1984, pages 661-664, XP000925591 * page 661, column 1, line 15 - line 16; table 2 *	1-8	
P,A	EP 0 921 190 A (ORIENTAL YEAST CO. LTD.; NAT. FOOD RESEARCH INST.) 9 June 1999 (1999-06-09) * examples 4,5 *	1-8	
	-----		TECHNICAL FIELDS SEARCHED (Int.Cl.7)
			C12N A21D
INCOMPLETE SEARCH			
<p>The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.</p> <p>Claims searched completely :</p> <p>Claims searched incompletely :</p> <p>Claims not searched :</p> <p>Reason for the limitation of the search: see sheet C</p>			
Place of search	Date of completion of the search	Examiner	
BERLIN	27 July 2000	Schönwasser, D	
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			
T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			



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INCOMPLETE SEARCH  
SHEET C

Application Number  
EP 00 40 0649

Claim(s) searched incompletely:  
1-8

Reason for the limitation of the search:

Present claim 1 and dependent claims relate to a yeast defined by reference to the following parameter: the ability to generate 110 ml of carbon dioxide or more per 40 g of dough containing 0.4 g of the yeast on a dry weight basis, the dough being confectionary or bread dough containing 40 parts by weight of sucrose per 100 parts by weight of flour.

The use of this parameter in the present context is considered to lead to a lack of clarity within the meaning of Article 84 EPC. It is impossible to compare the parameter the applicant has chosen to employ with what is set out in the prior art. The lack of clarity is such as to render a meaningful complete search impossible. Consequently, the search has been restricted to: a yeast with sugar tolerance being able to generate carbon dioxide in a confectionary or bread dough containing 40 parts by weight of sucrose per 100 parts by weight of flour.

ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.

EP 00 40 0649

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.  
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27-07-2000

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5801049	A	01-09-1998	NONE	
EP 0921190	A	09-06-1999	JP 11169180 A	29-06-1999

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82